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Title: **Mate-guarding by male mandrills (*Mandrillus sphinx*) is associated with female MHC-genotype**

Short title: **Male mate-guarding and female genotype**

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LAY SUMMARY

We examined whether male mate-guarding in mandrills varies with female genotype. Males were less likely to mate-guard females with specific genotypes which may be disadvantageous to offspring, and more likely to mate-guard females with genes that were different to their own, which would result in genetically diverse offspring.

ABSTRACT

Female choice for male major histocompatibility complex (MHC) genotype has been widely tested, but the relationship between male mating strategies and female MHC genotype has received far less attention. Moreover, few studies of MHC-associated mate choice test for the fitness effects underlying such choice. We examined mate-guarding by male mandrills, a species with intense male-male competition and female offspring care. We developed a statistical model based on 10 years of observations to describe how the probability a female is mate-guarded varies across her sexual cycle, among cycles and among females. We accounted for female rank, parity and maternal relatedness. We then tested whether the occurrence of mate-guarding is influenced by (i) MHC-dissimilarity, (ii) female MHC-diversity, and (iii) specific female MHC genotypes. Finally, we tested for associations between MHC variables and the ratio of neutrophils to lymphocytes in blood samples taken during routine captures. The best-fit models included either MHC-dissimilarity (males were more likely to mate-guard more dissimilar females, and there was some evidence of preference for intermediate MHC-dissimilarity), or a specific MHC supertype. Four of 11 supertypes investigated were influential and one had a strong negative influence on mate-guarding. We found some evidence that the MHC genotype that attracted the least mate-guarding was disadvantageous in terms of immune function. However, we did not find evidence that MHC-diversity was related to immune function. These results suggest that highly competitive males modify their mating behaviour based on female MHC genotype, and a possible fitness benefit to mate choice for specific genotypes.

KEYWORDS: major histocompatibility complex; dissassortative mating; good genes; mate choice; mate preference; sexual selection

INTRODUCTION

The major histocompatibility complex (MHC) plays a key role in the vertebrate immune system (Klein, 1986). As a result, it has been the focus of extensive research in the context of mate choice for genetic benefits (Milinski 2006; Eizaguirre et al. 2009; Trowsdale 2011). Three major hypothesised benefits of MHC-associated mate choice have been proposed: choice for MHC-dissimilar mates, choice for MHC-diverse mates, and choice for mates with specific MHC genotypes (Piertney and Oliver 2006). Choice for MHC-dissimilar mates may facilitate inbreeding avoidance and increase genome-wide heterozygosity in offspring or increase their immunological capacity (Doherty and Zinkernagel 1975; Penn and Potts 1999). However, excessive differences may have negative consequences if there are immune system trade-offs associated with too many MHC alleles, or if locally adapted gene complexes are disrupted, leading to choice for intermediate MHC-diversity in offspring (Wegner et al. 2003; Woelfing et al. 2009). Choice for an MHC-diverse mate may increase offspring heterozygosity without reference to the chooser's own genotype (Reusch et al. 2001), or increase the chance of offspring inheriting rare, beneficial alleles (Apanius et al. 1997). Finally, choice for mates with specific MHC genotypes may provide offspring with resistance to prevalent parasites (Penn and Potts 1999). Choice for such 'good genes' confers an additive fitness benefit, regardless of the presence or absence of other genes.

All three of these hypotheses relating mate choice to MHC genotype are supported in the literature. A recent quantitative review of 48 empirical studies of 27 animal species including fish, amphibians, reptiles, birds and mammals found support for female choice for MHC-diversity, choice for dissimilarity at multiple loci regardless of which sex chooses, and preliminary support for choice for an optimal level of MHC-diversity in offspring (Kamiya et al. 2014). Choice for specific loci in a mate has also received support in empirical studies (e.g., Eizaguirre et al., 2009; Ekblom et al., 2004). There is good evidence that specific MHC genotypes are related to resistance to particular pathogens (Schad et al. 2005; Schwensow et al. 2007; Trachtenberg et al., 2003), and MHC-diversity is linked to fitness (Wegner et al. 2003; Kurtz et al. 2004; Bonneaud et al. 2006; Kloch et al. 2010), providing indirect support for the fitness benefits of MHC-associated mate choice. However, few studies have investigated whether preferred MHC genotypes are linked to fitness in the same study population (Setchell and Huchard 2010).

Studies of MHC-associated mate choice are based on preference experiments, examine the mating outcomes of naturally paired animals in the wild or under controlled settings, or relate reproductive success to MHC variability (Kamiya et al. 2014). They focus overwhelmingly on either female choice behaviour, or on parentage, which results from a combination of male and female strategies. This focus on female choice is explained by classical models of sexual selection, which focus on male-male competition and female choice (Gowaty and Hubbell 2009). However, both theory and empirical studies suggest that both sexes may be choosy, and that male choice can occur even where traditional theory would not predict it, including in polygynous species and those with no male parental care (Clutton-Brock 2007; Bonduriansky 2009; Clutton-Brock 2009; Edward and Chapman 2011). To date, however, the question of MHC-associated mate choice in males has been largely overlooked.

The few studies of MHC-associated mate choice in males have produced mixed results. Males of the sex-role reversed broad-nosed pipefish (*Syngnathus typhle*) chose MHC-dissimilar females over MHC-similar females in olfactory mate choice experiments, but not when visual cues were available (Roth et al. 2014). Males of another role-reversed species, the potbellied seahorse (*Hippocampus abdominalis*), showed no preferences based on female MHC genotype in experiments testing for preferences for olfactory and visual cues and mated randomly with respect to MHC dissimilarity, based on mating success (Bahr et al. 2012). Among species with conventional sex roles, captive male chinook salmon (*Oncorhynchus tshawytscha*) also mated randomly with respect to female MHC genotype (Neff et al. 2008). Red junglefowl (*Gallus gallus*) showed cryptic male preference under some experimental circumstances (Gillingham et al. 2009). Males showed no overt behavioural mating preferences when presented with a choice between MHC-similar and MHC-dissimilar females, but allocated more sperm to the more MHC-dissimilar of two sequentially presented females, although not when simultaneously presented with an MHC-similar and an MHC-dissimilar female (Gillingham et al. 2009). Male laboratory mice showed choice for MHC-dissimilar females in some studies (e.g., Yamazaki et al., 1976), but not in others (Eklund et al. 1991). Men preferred the scent of MHC-dissimilar women and of women with common MHC alleles (Thornhill et al., 2003; Wedekind & Füri, 1997). Finally, both sperm numbers and testosterone levels were higher when male horses (*Equus caballus*) were exposed to MHC-dissimilar mares than when the same males were exposed to MHC-similar mares, suggesting that female MHC genotype influences male reproductive strategy, although sperm velocity was not affected (Burger et al. 2015). Together, these studies provide limited support

for the hypothesis that female MHC genotype influences male reproductive strategies, and suggest that this is a question that merits further attention.

Mandrills (*Mandrillus sphinx*) are an archetype of classical sexual selection theory. Males are far larger than females, and are resplendently colourful (Darwin 1871). Mandrills live in large multi-male, multi-female groups, in which females care for the offspring. Alpha males account for 94 % of peri-ovulatory mate-guarding activity and sire 69 % of offspring (Setchell et al. 2005a). In line with classical sexual selection theory, male-male competition is intense (Setchell et al. 2006), and females are choosy (Setchell 2005). However, mating effort is costly for males who receive serious wounds in the competition for top rank, particularly when sexually attractive females are available (Setchell et al. 2006). Alpha males attempt to monopolise access to receptive females by mate-guarding them for one to several days, during which time the male cannot mate-guard other available receptive females (Setchell et al. 2005a; Setchell and Wickings 2006). This suggests that the capacity to mate is less than the available mating opportunities, a condition which selects for male mate choice (Edward and Chapman 2011). Alpha males may exercise choice in terms of whether or not they mate-guard a female on any one day, and by choosing which female to mate-guard on days when more than one receptive female is available (Setchell et al. 2005a; Setchell and Wickings 2006). Although mate-guarding is not an unambiguous proxy for male mate choice behaivour, as it may be influenced by female behavior, the study of natural mating behavior allows us to measure the strength of the effect of different female traits on mate-guarding.

A previous study of sexual selection for MHC genotype in mandrills compared the MHC genotype of the sire of each infant with that of all other potential sires present at the conception of each offspring (Setchell et al. 2010). These analyses revealed that sires were more MHC-dissimilar to the mother and more MHC-diverse than the other males available at the time of conception, but that specific MHC genotypes did not predict the identity of the sire (Setchell et al. 2010). These analyses of mating outcomes cannot fully disentangle male and female strategies, but the comparison of actual sires with all potential sires suggests that the observed patterns result from female choice for male MHC genotype. Here, we examine pre-copulatory mate-guarding behaviour by males. We combine the same MHC genotype data with 10 years of daily records of female cycle status and the occurrence of mate-guarding by alpha males to test whether male mate-guarding is associated with female MHC genotype. Our dataset, based on multiple contributions from each male and female, shows a

highly stochastic, non-linear temporal pattern of mate-guarding. To adequately describe the data we developed a statistical model that estimates changes in the probability a female is mate-guarded across her sexual cycle and how the probability of mate-guarding varies among cycles. Our model-building approach focused on finding the most informative model rather than traditional p-values. First, we identified the non-MHC traits of individual females that provide the most parsimonious explanation of male mate-guarding in mandrills. Previous studies suggest that these are female dominance rank and parity (Setchell and Wickings, 2006). We also included maternal relatedness to account for a possible 'rule-of-thumb' to avoid mating with close kin in mandrills, which relates to their matrilineal society. Next, we accounted for these factors, and tested the hypotheses that incorporating MHC-genotype could further explain the data. Based on the three hypotheses relating MHC-genotype to mate-guarding, we predicted that the occurrence of mate-guarding is influenced by (i) MHC-dissimilarity between the male and female, (ii) female MHC-diversity, and (iii) specific MHC genotypes in females. In each case, we predicted that incorporating genotype information would improve the fit of the model. If the relationship between male mate-guarding and female MHC genotype parallels that found in our earlier study of female choice for male MHC genotype (Setchell et al. 2010), we predicted that mate-guarding would increase with MHC-dissimilarity in the dyad, and with MHC-diversity in the female, but would not relate to specific MHC genotypes in the female. Finally, we tested the hypothesis that MHC-genotype is associated with fitness, which underlies models of mate choice for MHC genotype, by testing the prediction that MHC genotype and measures of immune function are related in our study population.

METHODS

Study population

We studied the semi-free-ranging colony of mandrills housed at the Centre International de Recherches Médicales, Franceville, (CIRMF), Gabon. We used daily records of female cycle status (Setchell and Wickings 2004) and male mate-guarding (Setchell et al. 2005a) for the period 1996-2005. Group sizes ranged from 21 to 104 animals (mean+/-SEM 45+/-3) during this period. We extracted information on dominance rank (based on avoidance interactions) (Setchell et al. 2005b), female parity (nulliparous vs. parous) and matriline membership from

long-term colony records.

Cycling female mandrills show highly visible sexual swellings during the follicular phase of the menstrual cycle (Setchell and Wickings 2004). Peak swellings coincide with peak sexual activity, and occur at or after the day of peak urinary estrone conjugates (Phillips and Wheaton 2008). Rapid detumescence coincides with the post-ovulatory rise in progesterone and continues during the luteal phase (Phillips and Wheaton 2008). Conception is most likely during 5 days before detumescence in a closely-related species (Gesquiere et al. 2007). We numbered cycle days according to the day of detumescence, with the first day of detumescence termed day 0.

Systematic, focal observations of sexually receptive females are not possible under colony conditions. We, therefore, used the occurrence of mate-guarding as a behavioural estimate of male attempts to secure unique access to a receptive female. Mate-guarding is a readily observed, unambiguous behaviour where a male follows a female closely and persistently, interacts with her sexually, and attempts to prevent other males from doing so (Setchell et al. 2005a). It is commonly used as a measure of mating success in baboons (Bercovitch 1986; Bulger 1993; Altmann 1996; Weingrill et al. 2000; Alberts et al. 2003) and is closely related to reproductive success in mandrills (Setchell et al. 2005a), rhesus macaques (*Macaca mulatta*, Berard et al. 1994; Bercovitch 1997), long-tailed macaques (*M. fascicularis*, de Ruiter et al. 1994; Engelhardt et al. 2006) and Japanese macaques (*M. fuscata*, Matsubara 2003). We recorded mate-guarding daily as a binary variable based on behavioural observation sessions made twice daily (approx. 10h00-11h30 and 15h30-17h30) from a tower overlooking the enclosures. We concentrated on alpha males, who account for 77-100 % of peri-ovulatory mate-guarding activity in a mating season (Setchell et al. 2005a) and can be assumed to have free choice of female. Mate-guarding dyads did not change between morning and afternoon sessions and ad libitum observations suggest that mate-guarding continues at night (Setchell et al. 2005a). We therefore assumed that mate-guarding continued outside observation periods.

Up to 16 females showed sexual swellings on any one day during the study period, but a maximum of six females were simultaneously within 6 days of detumescence, when mate-guarding peaks (Setchell et al. 2005a). No other female was within 6 days of detumescence on 65 % of periovulatory days.

MHC genotyping

We genotyped all animals for which we had sufficient DNA for MHC-DRB, a highly variable group of MHC class II loci (details in Abbott et al. 2006; Setchell et al. 2010). Ideally, studies should survey a larger region of the MHC than this, but this requires a level of knowledge of MHC structure that is lacking for most non-model organisms. Fortunately, the MHC region is characterized by strong linkage disequilibrium (Kelley et al. 2005), meaning that relatively small segments of the MHC provide valuable information about the larger complex.

We focus on the number of different sequences possessed by an individual as a measure of MHC-diversity, without making any assumptions about the number of loci involved, because the complexity of the primate MHC makes a locus-specific approach to MHC characterisation impractical. We excluded sequences that were not expressed, or which included a stop-codon (Setchell et al. 2010). We also characterised variants and pooled them into 11 functionally similar supertypes (Doytchinova and Flower 2005). Supertypes are groups of sequences that share peptide-binding motifs and are therefore thought to be functionally similar. They also collapse large numbers of sequences, each present in only a few animals, into a smaller number of variables, more suitable for use in statistical analyses (Schwensow et al. 2007). Mandrills in this study possessed two to seven MHC sequences (n = 47, mean+/-SD 3.9+/-1.1) and two to six MHC supertypes (mean+/-SD 3.6+0.9).

Immune function

We quantified general immune function as the ratio of neutrophils to lymphocytes based on differential leukocyte counts obtained from long-term records of haematological analyses conducted at CIRMF following annual captures of the mandrills (details in Setchell et al., 2006). An increase in this ratio indicates a reduction in immune function (Kim et al. 2005).

Statistical analyses

In total, our data span 4183 female observation days across 9 years for 36 female mandrills over 249 cycles (mean+/-SD 6.9+/-6.0 cycles per female, median 5, range 1-28), and mate-

guarding by 11 alpha males (mean+/-SD 22.6+/-16.0 cycles per male, median 15, range 7-56) (Fig. 1). We developed a likelihood function that described the probability a male would be observed mate-guarding a female on a given day throughout her cycle (Fig. 2). The likelihood incorporated female traits, maternal relatedness, and MHC-related covariates, and accounted for non-independent sampling, minimizing the chance of incorrect inference that can occur due to pseudo-replication.

Although many evolutionary ecologists are familiar with the use of linear random effects models to account for replication in a dataset, such an approach cannot account for the highly non-linear aspects of our data. Rather than simply assume a logistic relation between the probability a female is observed to be mate-guarded by a male, p, and the number of days before detumescence, t, we instead proposed the relation

$$p(t) = \frac{p_{\max} x(t)}{1 - w + w x(t)^{1/w}}, \qquad (1)$$

where

$$x(t) = \left(\frac{t + \varepsilon}{t_{\max} + \varepsilon} \right)^{1+\gamma}, \qquad (2)$$

and $t_{\max}$ is the day when mate-guarding is most likely, when mate-guarding occurs with probability $p_{\max}$. Mate-guarding is positively influenced by $0 < w < 1$, γ is a scaling parameter, and $\varepsilon > 0$ allows mate-guarding on day 0.

Our analyses tested whether $p_{\max}$ (the probability of mate-guarding on the day of peak mate-guarding) and w (related to the duration of mate-guarding) were influenced by female traits (rank, parity and genotype) or some male-female relationship (e.g., shared matriline, MHC differences). There is no biological reason to expect $t_{\max}$ to vary with the variables of interest.

Suppose female cycles are associated with the four covariates: $\mathbf{X} = \{X_1, X_2, X_3, X_4\}$. We set covariate $X_1 = 1$ or 0 to indicate whether or not the female and male are of the same matriline. Covariate X_2 indicates the rank of the female at the time of the cycle: 1 (high: upper quartile), 2 (mid: middle 50%) and 3 (low: lower quartile). Covariate $X_3 = 1$ or 0 indicates female parity (1 = parous, 0 nulliparous). Data on these three covariates are presented below the x-axis in Fig. 1 (X_1 = M, X_2 = R, X_3 = N). Covariate X_4 describes the genetic information associated with the cycle. Depending on the hypothesis tested, X_4 describes one of three

genetic measures: (1) the number of MHC sequences that differed between the male and female (MHC-dissimilarity, $X_4 = G_1$); (2) the total number of MHC sequences possessed by the female (MHC-diversity, $X_4 = G_2$); or (3) if the female possessed a specific MHC supertype ($X_4 = G_3$). We only considered one of these genetic predictors at a time because they were often correlated. Similarly, as possession of some of the supertypes are correlated (Table S3) we modelled the effect of each supertype in isolation.

For a model that considers all four covariates

$$p_{\max}(\bar{p}_{\max}, \mathbf{X}_j, u) = T\left[\bar{p}_{\max}, u + \sum_{i=1}^{3} a_i \left(\frac{X_{i,j} - \bar{X}_i}{s_i}\right) + a_{4,1}\left(\frac{X_{4,j} - \bar{X}_4}{s_4}\right) + a_{4,2}\left(\frac{X_{4,j} - \bar{X}_4}{s_4}\right)^2\right] \quad (3)$$

and

$$w(\bar{w}, \mathbf{X}_j, r, u) = T\left[\bar{w}, r\,u + \sum_{i=1}^{3} b_i \left(\frac{X_{i,j} - \bar{X}_i}{s_i}\right) + b_{4,1}\left(\frac{X_{4,j} - \bar{X}_4}{s_4}\right) + b_{4,2}\left(\frac{X_{4,j} - \bar{X}_4}{s_4}\right)^2\right], \quad (4)$$

where $X_{i,j}$ is the value of the i-th covariate observed during cycle j, and $\bar{X}_i$ and s_i are the mean and standard deviation of covariate i across the J observed cycles (Table S1). The α and β parameters describe the relative effect sizes of the covariates, u describes the effect of unknown covariates on $p_{\max}$, and ρ describes how this effect correlates with w. T is a transformation ensuring that $p_{\max}$ and w remain bounded: $T[p,v] = \exp(Z(p,v))/(1+\exp(Z(p,v)))$, where $Z(p,v) = \ln(p/(1-p))+v$. This transformation explicitly accounts for the fact that variation in the expected mate-guarding frequency among females will have a skewed distribution. We allow the genetic predictors, X_4, to have a quadratic effect on mate-guarding, except when it describes the presence of a specific supertype ($X_4 = G_3$).

We did not include the number of simultaneously cycling females in our model as it is unclear how it would systematically affect the probabilities associated with male mate-guarding behaviour (Setchell and Wickings 2006). Instead, we used the random variate, u, to account for cycle-specific differences due to unknown variables like the distribution of male and female reproductive states at a given time.

Figure 1 illustrates the degree of temporal non-independence in our data. For example, the first seven columns of observations are associated with a specific male-female dyad. For two of the female cycles the male was never observed to mate-guard the female, whereas for two

other cycles he mate-guarded her over five consecutive observation days. Two other cycles show some day-to-day switching back and forth between mate-guarding and non-mate-guarding. Thus, if a male mate-guarded a female during her cycle it was often for a number of consecutive days but the amount of mate-guarding he adopted varied considerably between her cycles. This high variation in mate-guarding intensity within male-female dyads suggests that the female cycle is a more appropriate scale for independence in our statistical model. Not surprisingly, model fitting resulted in much poorer fits when the model only assumed independence among dyads (not presented here). We implemented independence among cycles by applying the following likelihood function:

$$L(\theta) = \prod_{j=1}^{J} \int_{u=-\infty}^{\infty} N(u \mid 0, \sigma) \prod_{i=1}^{I_j} Y\left[y_{i,j}, p\left(t_{i,j} \mid p_{\max}(\bar{p}_{\max}, \mathbf{X}_j, u), w(\bar{w}, \mathbf{X}_j, r, u)\right)\right] du \qquad (5)$$

where J is the total number of female cycles observed, I_j is the number of observation days during cycle j, $y_{i,j} = 1$ if mate guarding was observed during the i-th observation of cycle j (0 otherwise), $t_{i,j}$ is the day of the observation, and $Y[y,p] = yp+(1-y)(1-p)$. $N(u|0,\sigma)$ is the normal distribution with mean 0 and standard deviation σ, representing random variation among cycles that cannot be accounted for by the measured covariates (see SI for additional details).

We sought evidence of genetic effects on mate-guarding as follows. First, we used Akaike's Information Criterion (AIC) to identify the combination of non-MHC covariates X_1-X_3 that provided the most parsimonious explanation of the data. We then added the three versions of the MHC covariate X_4 to the best AIC model (Table S1) and again used AIC to determine if MHC information improved the model further. This approach ensured that we only concluded genetic effects after non-genetic effects had already been accounted for. We selected models as being informative if they had an AIC score within six of the lowest calculated and no simpler nested model had a lower AIC score. A threshold of six approximates to a 95% chance of retaining the model having the lowest expected Kullback-Leibler distance in a range of ecological studies (Richards, Whittingham, & Stephens, 2011; Richards, 2008).

We developed and fitted linear mixed-effects models to the immune function data to test whether they were correlated with each supertype or with MHC-diversity. We included a random effect to account for repeated samples among females, and a factor to account for

seasonal variation (Setchell et al. 2007). We used likelihood ratio tests to compare fits with and without the MHC variable of interest to assess their predictive capacity.

We had immune function data (ratio of neutrophils to lymphocytes) for 146 individuals (73 females, 73 males, 1-19 values each) throughout the year, spanning 1983-2004 (Setchell et al. 2006). We included animals of both sexes, as mate choice for MHC benefits concerns the potential genotypes of offspring of both sexes. We log-transformed the ratios, as it improved the fit of the model, and modelled their expected value according to

$$\mu(x_1, x_2, m) = \bar{\mu} + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \alpha_m, \quad (6)$$

where x_1 = 1 or 0, indicating if the animal is male or female, x_2 = 1 or 0, indicating if the animal possesses the supertype of interest, and m (= 1-12) is the month of the sample. We included an interaction between sex and supertype, as the sexes differ in immune function (Klein 2004). The set of parameters $\boldsymbol{\alpha} = \{\alpha_1 \ldots \alpha_{12}\}$ sum to zero and account for seasonal variation in immune status. The likelihood of the data is

$$L(\bar{\mu}, \beta_1, \beta_2, \beta_{12}, \boldsymbol{\alpha}, \sigma_b, \sigma_w) = \prod_i \int_u N(u; 0, \sigma_b) \prod_j N(y_{ij}; \mu(x_{1i}, x_{2i}, m_{ij}) + u, \sigma_w)\, du \quad (7)$$

where y_{ij} is the log-transformed immune function value taken during the jth sample of individual i m_{ij} is the month of the sample, and N is the probability density of the normal distribution. The integration variable u in Eq. 7 accounts for repeated sampling among individuals, and the parameters σ_b and σ_w quantify the degree of variation in the data between and within individuals.

For each supertype we used a likelihood ratio test (LRT) to test the sex by supertype interaction (i.e., we compared the fits when β_{12} was estimated from the data and when it was set to zero). If the test was not statistically significant we removed the interaction term and performed another LRT to test whether the parameter quantifying the supertype's main effect, β_2, was non-zero.

We used a similar model to test whether immune function was related to the total number of MHC sequences in an individual. We again used LRTs to test for sex and MHC effects (linear and quadratic). To simplify the interpretation of our analyses, when testing the interaction between total MHC sequences and sex, we removed the quadratic term, and when testing for a quadratic effect of total MHC we removed the interaction term.

Ethics statement

This study was approved by the Comité d'Ethique Paris Sud and complied with animal care regulations and applicable national laws in Gabon. Blood sampling occurred during routine annual veterinary examinations; observations of sexual behaviour are entirely non-invasive.

RESULTS

Models without MHC effects

Our statistical model matched the mate-guarding data closely. The probability of mate-guarding peaked just before female sexual swellings detumesced, when females are most likely to be fertile, and was very low at $\geq$10 days before detumescence (Fig 2), when conception is very unlikely (Wildt et al. 1977; Gesquiere et al. 2007). The best AIC model without incorporating MHC genotype included female rank and whether or not the female and male were of the same matriline (Model R+M; Table 1). Model M was the only other model selected according to AIC (Table 1). Thus, we found strong evidence of a maternal effect and some evidence that female rank influenced the probability of a female being guarded. Given evidence of M and R effects we next asked the question: can the addition of MHC-related variables improve the models further according to AIC (i.e., can the residual variation be explained by MHC)?

Models incorporating MHC effects

In total we fit 15 additional models by adding genetic information to model R+M (Table 1). Overall, the best AIC model was M+R+G_3 when the presence/absence of *supertype S1* was considered; this model was much more parsimonious than model R+M (AIC was reduced by 7.5; Table 1). Five additional models were also considered as candidates for the most parsimonious description of the data according to AIC (Table 1). None of these models incorporated female MHC-diversity (G_2). The models that included a linear or a quadratic term associated with MHC-dissimilarity (G_1) produced AIC values that were not much larger than the best AIC model (Table 1). Plotting the best parameter estimates for the quadratic model, which had a lower AIC value, indicated that males were more likely to mate-guard

females when they had an intermediate (6-8) number of different MHC sequences, in comparison to pairs with few (1-5) or many (9-11) different sequences (Fig 3). However, evidence for a quadratic term was not strong as the model that ignored it was also selected (Table 1); the model that ignored this term ($R+M+G_1$) predicted an increase in mate-guarding with increasing MHC-dissimilarity. For four of the 11 supertypes, knowing if the female possessed the supertype resulted in the associated model being selected according to AIC (Table 1). The most influential supertype, S1, was highly negatively correlated with mate-guarding (Table 1, effect illustrated in Fig 3a). Although the analysis also suggested that S5 was also influential, its prevalence was positively correlated with the presence of S1 (Supplementary Table S3), which was more strongly related to mate-guarding, suggesting that avoidance is more likely due to the presence of S1. Possession of S4 and S8 also provided parsimonious explanations of the data but substantially less so than S1 (Table 1).

The model that incorporated MHC supertype S1 and the two models that included MHC-dissimilarity had similar AIC values. Patterns of mate-guarding predicted by these models suggest that these MHC-related variables had similar effect sizes on male mate-guarding behaviour to the known influence of female rank (Table 1, compare panels in Fig 3). The effect of both genetic variables was largely due to differences in the overall duration of mate-guarding, rather than a greater probability of mate-guarding on the last few days before detumescence (Fig 3).

MHC genotype and immune function

When we examined the relationship between MHC genotype and immunity, we found no significant interactions between sex and MHC genotype, so we removed the interaction term. Supertype S1 (the supertype which was highly negatively correlated with mate-guarding) was most strongly and negatively correlated with the neutrophil to lymphocyte ratio (Table 2); mandrills with supertype S1 exhibited a 16.8% reduction in immune function compared with those that did not have this supertype. The result for S1 is statistically significant at the 5% level when considered in isolation (Table 2), but not when corrected for multiple comparisons (Bonferroni threshold 0.05/11 = 0.0045). None of the remaining supertypes (which were less related or unrelated to male choice) were significantly related to immune function (Table 2). We found no support for a relationship between MHC-diversity and immune function (Table 3).

DISCUSSION

We modeled naturally occurring mate-guarding behavior by alpha male mandrills, accounting for non-genetic influences (rank and parity), and the possibility that mandrills employ a simple rule-of-thumb not to mate with members of their own maternal lineage. The results for the effect of dominance rank on male mate-guarding are consistent with previous analyses, but those for female parity and maternal relatedness are not (Setchell and Wickings 2006). These differences are likely to reflect the fact that earlier analyses investigated only the six days before detumescence of the sexual swelling, and did not account for variation between these days. We then tested whether including information about female MHC genotype and about MHC-dissimilarity further improved the fit of our model. We found support for two of the three proposed models of MHC-associated mate-guarding (mate-guarding was associated with MHC-dissimilarity and with specific genotypes), but not the third (mate-guarding was not associated with MHC-diversity). The best AIC models included either MHC-dissimilarity between male-female pairs, or one of four specific MHC supertypes, one of which (S1) had a very strong negative influence on mate-guarding. While the latter result, for specific genotypes, results from multiple tests comparing models with and without each of 11 different supertypes, our finding that four of 11 supertypes were selected by AIC, makes it unlikely that the observed results are the result of Type 1 error. In contrast, models that added information about female MHC-diversity were not selected. Overall, our findings suggest that aspects of MHC genotype are as important an influence on mate-guarding as female rank, suggesting that additive genetic effects may be as important to male mandrills as non-additive effects.

We also found some suggestion that mandrills with supertype S1 may have a poorer immune system, providing indirect evidence of an adaptive reason why males might prefer to mate-guard females without this supertype. Although MHC-dissimilarity influenced mate-guarding by males, suggesting selection for MHC-diverse offspring, we found no evidence in support of a fitness benefit associated with MHC-diversity (MHC-diversity was not related to the measure of immune function we used, the ratio of neutrophils to lymphocytes).

Mate-guarding and MHC-dissimilarity

We found statistical support for both a linear and a quadratic effect of MHC-dissimilarity on male mate-guarding. Inspection of the parameter estimates for model ($R+M+G_1+G_1^2$) suggested that males were more likely to mate-guard females that were of intermediate MHC-dissimilarity. However, model ($R+M+G_1$) was also selected according to AIC and this model predicted that males were more likely to mate-guard females with increasing MHC-dissimilarity. These results suggests that mandrills may be choosing for optimally different partners or partners with maximal differences. The predictions of model ($R+M+G_1+G_1^2$) are consistent with the hypothesis that mate choice acts either to avoid the costs of both inbreeding and outbreeding at a genome-wide level, or to pass on a combination of MHC genes to offspring that represents an optimal trade-off between the ability to recognise a breadth of antigens via increased MHC-diversity and disadvantages associated with high MHC-diversity (Nowak et al. 1992; Milinski et al. 2005; Woelfing et al. 2009). Choice for intermediate dissimilarity has also been reported for some female fish (Reusch et al. 2001; Milinski et al. 2005; Forsberg et al. 2007), and birds (Bonneaud et al. 2006; Baratti et al. 2012). The predictions according to the alternative parsimonious model ($R+M+G_1$) are similar to those of our earlier study comparing sires with all potential sires, in which we found selection for maximally, rather than optimally, MHC-dissimilar mates (Setchell et al. 2010).

We found no link between MHC-diversity and the ratio of neutrophils to lymphocytes, a measure of immune function. However, other studies show support for a link between optimal MHC-diversity and fitness. For example, MHC-diversity is linked with improved parasite resistance in fish (Wegner et al. 2003; Kurtz et al. 2004), birds (Bonneaud et al. 2006) and mammals (Kloch et al. 2010) and with higher reproductive success in fish (Lenz et al. 2013). Improved measures of immunocompetence (e.g., Drury 2010) and measures of parasite infection are needed to test for a fitness benefit to mate choice for MHC-diversity in offspring in non-human primates (Setchell and Huchard 2010).

The relationship between MHC-dissimilarity and male mating effort was evident even though we accounted for shared matriline, suggesting that avoidance of close maternal kin alone is insufficient to explain the relationship with MHC differences. Mandrills are likely to be able to distinguish maternal kin via familiarity, due to their matrilineal social system. Despite their polygynandrous mating system, they may also be able to distinguish paternal kin

(Charpentier et al. 2007), although evidence to date suggests that the influence of maternal kinship on social behaviour is far stronger than that of paternal kinship in cercopithecine primates (Widdig 2013). The relation between male behaviour and MHC-dissimilarity predicted by model ($R+M+G_1$) may, therefore, be explained by avoidance of mating with paternal kin. Moreover, such paternal kin discrimination may itself be mediated via MHC-based phenotype matching (Charpentier et al. 2007; Widdig 2007). Chemical communication may provide a proximate mechanism underlying both male choice based on MHC-differences and paternal kin discrimination. Odour similarity is strongly related to MHC-similarity in mandrills, suggesting that males may use olfaction to compare their own genotype with that of potential mates (Setchell et al. 2011).

Mate-guarding and specific MHC genotypes

Male mandrills were more likely to mate-guard females who possessed specific MHC genotypes. These findings support the hypothesis that mate choice favours an additive genetic benefit to offspring (Apanius et al. 1997). Such choice has also been reported for female birds (Ekblom et al. 2004) and fish (Eizaguirre et al. 2009) but contrasts with the results of our earlier analyses, which found no effect of particular MHC supertypes on which male sired an offspring, providing no support for the hypothesis that females choose for specific MHC genotypes in males.

By reducing their mating effort when females possess supertype S1, male mandrills may select against 'bad genes', as possession of this supertype is associated with decreased immune function in our study population. The lack of strong statistical evidence linking supertypes with immune function may not be too surprising given that many other factors also influence immune function (Setchell et al., 2007) and that some supertypes are rare, reducing statistical power. Possible proximate mechanisms by which males may discriminate between females with different specific MHC genotypes include colour signals (Setchell et al. 2006), odour (Setchell et al. 2011) and variation in sexual swelling size and shape (Huchard et al. 2010).

Mate-guarding and MHC-diversity

We found no effect of female MHC-diversity on the occurrence of mate-guarding. This contrasts with the results of earlier analyses of the same study population during the same period, which found that the actual sire of an offspring was more MHC-diverse than other potential sires were (Setchell et al. 2010). This advantage to MHC-diverse males is unlikely to be a result of a relationship between male vigour and reproductive success, as the analyses accounted for the effects of male-male competition. Thus, it appears that female mandrills choose MHC-diverse males, but that male mate-guarding is not biased towards MHC-diverse females. The reasons underlying mate choice for MHC-diversity are as yet unclear, but choice for an MHC-diverse mate may maximise offspring heterozygosity without reference to the chooser's own genotype (Reusch et al. 2001), or increase the chance of offspring inheriting rare, beneficial alleles (Apanius et al. 1997). The explanation for the possible sex differences is also unclear, but it is possible that males have no way of detecting MHC diversity in females, as female odour does not reflect MHC diversity, although male odour does (Setchell et al. 2011).

Conclusions

Whether and why males choose to mate with particular females is becoming an important question in evolutionary ecology (Edward and Chapman 2011). However, although female choice for male MHC genotype has been widely tested (Kamiya et al. 2014), male choice for female MHC genotype has been relatively overlooked. Moreover, few studies of MHC-associated mate choice by either sex test for the fitness effects that such choice is predicated on (Setchell and Huchard 2010). In this study, we set out to determine whether including information about female MHC genotype and about MHC-dissimilarity improved the fit of our model of naturally-occurring mate-guarding behavior by alpha male mandrills, when we accounted for non-genetic influences (rank and parity), and the possibility that mandrills employ a simple rule-of-thumb not to mate with members of their own maternal lineage. We found that males were, in general, more likely to mate-guard females with higher MHC-dissimilarity and there was also some evidence that males preferred females of intermediate MHC-dissimilarity. We also found evidence that a specific MHC supertype had a strong negative influence on mate-guarding and this MHC genotype was disadvantageous in terms of immune function. Our observational approach cannot disentangle the strategies of the two sexes, as both male and female choice may influence male mate-guarding. Nevertheless, our results support the hypothesis that male mandrills show mate choice, a role traditionally

assigned to females. Moreover, our results suggest that mate-guarding is influenced by potential genetic benefits to offspring as strongly as it is by the direct benefits of female rank. These findings support the view that the classical dichotomous view of sex roles hampers our understanding of sexual selection and should be discarded in favour of a more nuanced understanding that does not limit choice to the sex that cares for the offspring (Clutton-Brock 2007; Bonduriansky 2009; Clutton-Brock 2009; Edward and Chapman 2011).

DATA ACCESSIBILITY

MHC sequence data are deposited in GenBank (accession numbers DQ103715–DQ103732, DQ103734–DQ103746, EU693911–EU693914).

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SUPPLEMENTARY MATERIAL

Table S1: Summary statistics for female covariates and male-female pair covariates

Table S2: Maximum-likelihood parameter estimates for the two best AIC models

Table S3: Spearman's rank correlations among 11 MHC supertypes (S) in mandrills (n = 37 individuals)

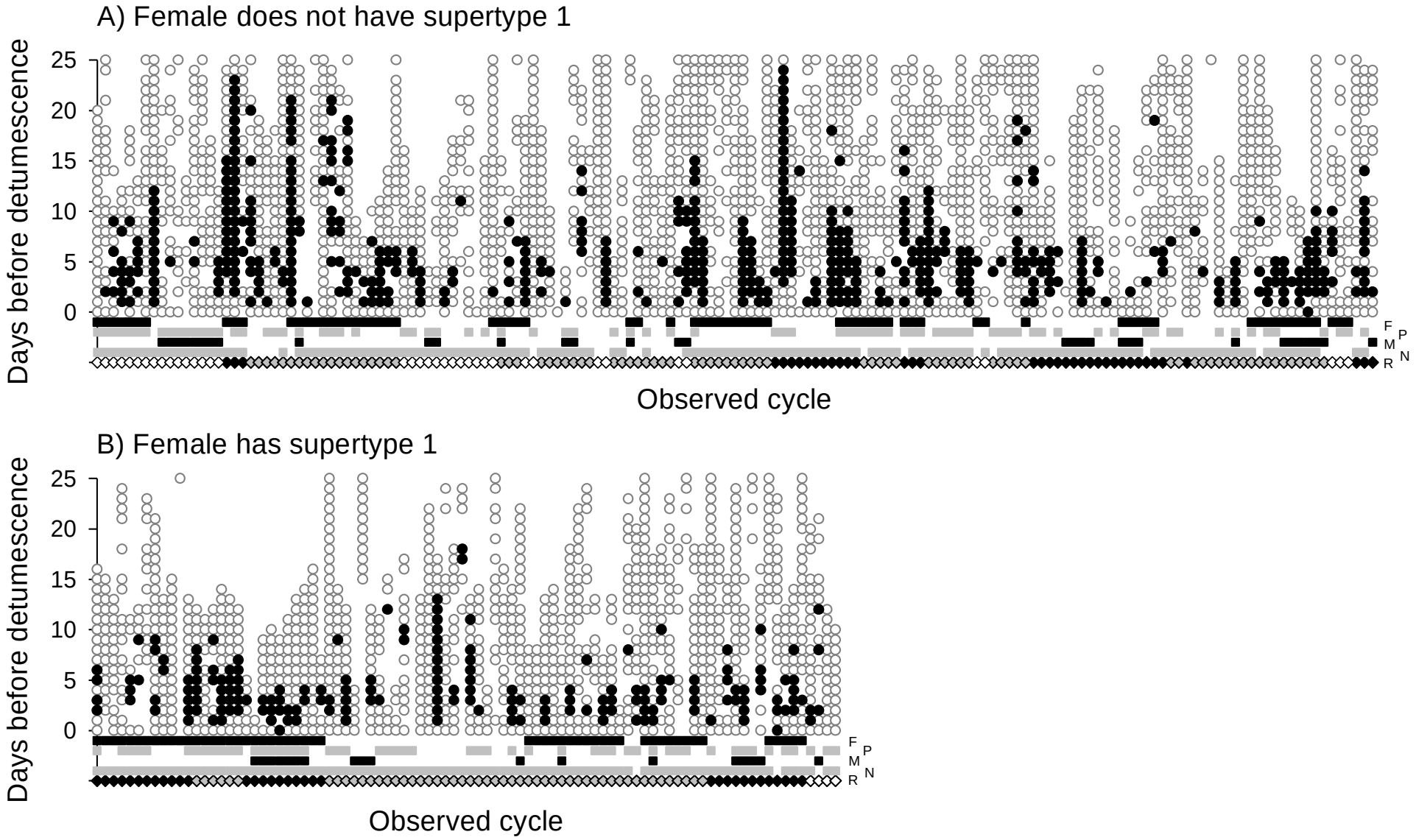
A) Female does not have supertype 1
Days before detumescence
25
20
15
10
5
0
F
M
R
P
N
Observed cycle
B) Female has supertype 1
Days before detumescence
25
20
15
10
5
0
F
M
R
P
N
Observed cycle

Figure 1: Observations of mate-guarding behavior by alpha male mandrills. The x-axis shows 249 reproductive cycles across 36 females that (A) do not and (B) do possess supertype 1 (S1). The positive y-axis shows the days of the cycle relative to sexual swelling detumescence, which coincides with the post-ovulatory rise in progesterone. Circles show days when the female was observed; open circles indicate females were not guarded and filled circles indicate guarding. Symbols immediately below the x-axis indicate the male-female pairings and three covariates associated with each reproductive cycle. Specifically, alternating black and white bars in row F distinguish individual females; alternating grey and white bars in row P distinguish individual male-female pairs. Black squares further below, in row M (covariate X_1 in our models), indicate that the male and female were from the same matriline; white squares indicate maternally-unrelated pairs. White squares in row N (covariate X_3) are nulliparous females; grey squares indicate parous females. Diamonds in row R (covariate X_2) indicate female rank at the time of observation: high (black), mid (grey) and low (white). Comparison of the data presented in panels A and B shows a propensity for females possessing S1 to be guarded less frequently than those without S1 (there are relatively more open circles in panel A than in panel B).

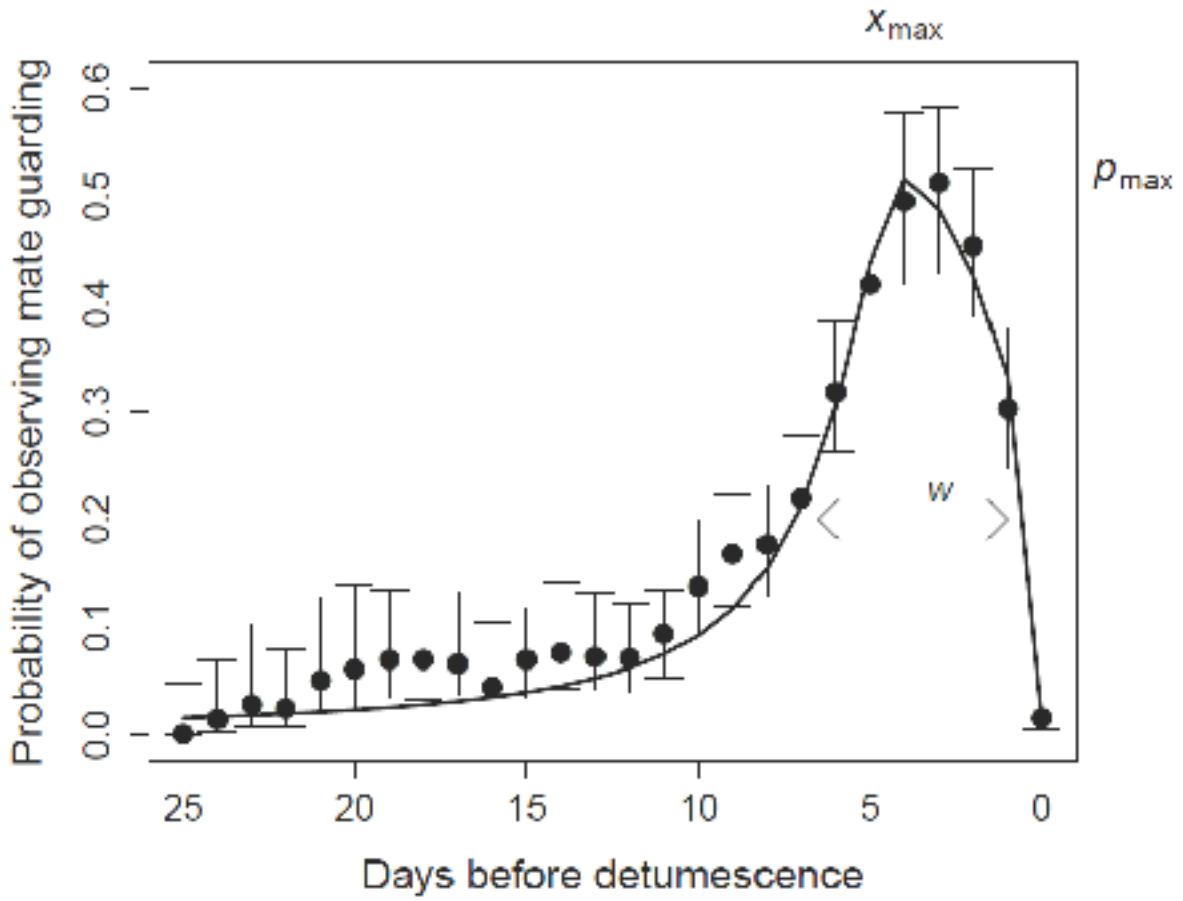

Figure 2: Observed and predicted probabilities that alpha male mandrills mate-guard females each day, relative to sexual swelling detumescence, which coincides with the post-ovulatory rise in progesterone. Error bars are estimated 95 % confidence intervals of the observed means. Solid line is the estimated probability of mate-guarding for a female of mean rank and mean relatedness to the alpha male. Plotting medians rather than means yields qualitatively similar patterns. We tested whether p_{max} (the probability of mate-guarding on the day of peak mate-guarding) and w (a measure of the duration of mate-guarding) were influenced by female traits (rank, parity and genotype) or some male-female relationship (shared matriline, MHC differences).

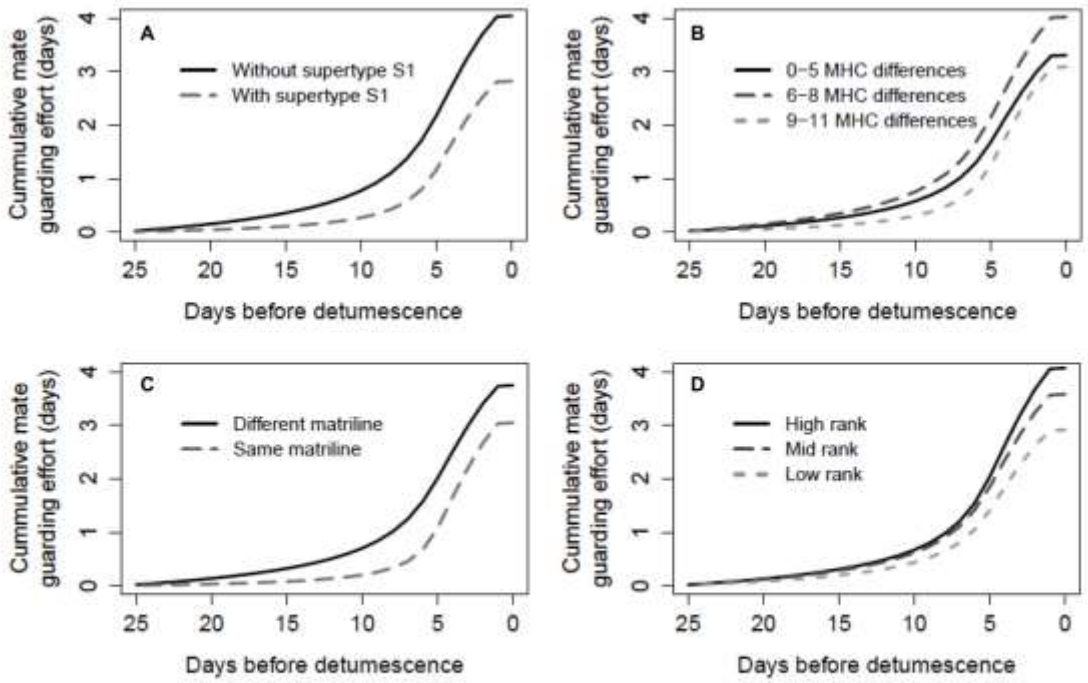

Figure 3: Estimated influence of potential MHC mate choice criteria on cumulative mate-guarding effort by alpha male mandrills. We calculated cumulative effort as the sum of the area under the predicted mate-guarding curve (see Fig. 2). Panel A depicts mean changes in effort throughout a female cycle according to the presence of specific MHC supertype S1 in females; panel B depicts mean changes according to MHC-dissimilarity. In B the predictions for each of the three categories are calculated by weighting according to the observed frequencies of MHC differences. When plotting the results we binned male-female pairs into those with few (1-5), intermediate (6-8), or many (9-11) different sequences, to achieve roughly equally sized categories; however, our statistical analyses use the exact number of different sequences, not these bins, which are for visualization purposes only. Panels C and D depict the effects of shared matriline and female rank, respectively, and illustrate relative effect sizes associated with non-genetic factors.

Table 1: Results of model selection testing whether MHC genotype variables (G_1-G_3) affected the intensity and duration of mate-guarding by male mandrills, when also accounting for female rank (R), whether or not the female and male are of the same matriline (M), and whether or not the female had previously given birth (nulliparity, N).

				ΔAIC	
				No MHC	Including
Model description	LL_{max}	***K***	**AIC**		**MHC**
Models without MHC effects					
Null	-1117.0	7	2248.0	8.3	15.8
R	-1114.7	9	2247.4	7.7	15.2
M	-1110.9	9	2239.9	0.2	7.7
N	-1116.6	9	2251.2	11.5	19.0
R+M*	-1108.9	11	2239.7	0.0	7.5
M+N	-1110.8	11	2243.5	3.8	11.3
R+N	-1114.4	11	2250.8	11.1	18.6
R+M+N	-1108.6	13	2243.2	3.5	11.0
Models incorporating MHC genotype effects					
G_1: *MHC-dissimilarity*					
R+M+G_1	-1103.7	13	2233.4		**1.2**
R+M+G_1+G_1^2	-1101.5	15	2233.0		**0.8**
G_2: *Female MHC-diversity*					
R+M+G_2	-1106.5	13	2239.0		6.8
R+M+G_2+G_2^2	-1106.4	15	2242.8		10.6
$G_{3,i}$: *Female has specific MHC supertype i*					
R+M+$G_{3,1}$	-1103.1	13	2232.2		**0**
R+M+$G_{3,2}$	-1108.4	13	2242.9		10.7
R+M+$G_{3,3}$	-1106.8	13	2239.6		7.4
R+M+$G_{3,4}$	-1105.3	13	2236.6		**4.4**
R+M+$G_{3,5}$	-1104.6	13	2235.1		**2.9**
R+M+$G_{3,6}$	-1108.0	13	2241.9		9.7

R+M+$G_{3,7}$	-1107.7	13	2241.4	9.2
R+M+$G_{3,8}$	-1105.4	13	2236.9	**4.7**
R+M+$G_{3,9}$	-1107.2	13	2240.4	8.2
R+M+$G_{3,10}$	-1107.6	13	2241.4	9.2
R+M+$G_{3,11}$	-1107.8	13	2241.5	9.3

* indicates the best AIC model without MHC effects; which for this analysis is model R+M. Note that model M is also in the selected set according to AIC. Given these results, models with MHC effects included female rank and matriline. LL_{max} denotes maximum log-likelihood and *K* is the number of estimated model parameters. A superscript 2 in the model description indicates a quadratic term (see Supplementary Material). ΔAIC is calculated when MHC variables are ignored and when MHC variables are included along with R and M. Bold ΔAIC values indicate models selected by AIC when MHC effects were also considered (see Methods). See Supplementary Tables S1 and S2 for a summary of the covariates and parameter estimates for the two best AIC models.

Table 2: Results of likelihood ratio tests investigating evidence for a relationship between each of 11 MHC supertypes and immune function (ratio of neutrophils to lymphocytes) in mandrills

	Sex by Supertype Interaction		*Supertype Main Effect*	
	G	***P***	***G***	***P***
Supertype 1	0.22	0.639	5.34	**0.021**
Supertype 2	1.93	0.165	0.87	0.350
Supertype 3	1.01	0.933	0.01	0.976
Supertype 4	0.01	0.942	0.65	0.419
Supertype 5	2.95	0.086	0.12	0.728
Supertype 6	0.02	0.901	1.88	0.170
Supertype 7	2.14	0.144	0.11	0.741
Supertype 8	0.54	0.465	0.02	0.900
Supertype 9	0.19	0.663	0.80	0.372
Supertype 10	0.15	0.700	1.01	0.314
Supertype 11	1.42	0.233	0.09	0.760

G is the likelihood ratio test statistic. All tests are associated with 1 df. *P* values less than 0.05 are in bold.

Table 3: Results of likelihood ratio tests testing the relationship between MHC-diversity and immune function (ratio of neutrophils to lymphocytes) in mandrills

	G	*P*
Sex by MHC Interaction	0.04	0.841
MHC (linear term)	2.13	0.144
MHC (quadratic term)	1.19	0.275

G is the likelihood ratio test statistic. All tests are associated with 1 df.